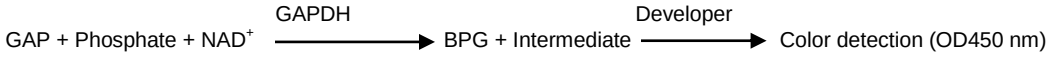
GAPDH Activity Assay Kit

rev. 9/14

(Catalog # K680-100; 100 assays; Store at -20°C)

I. Introduction:

GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase; EC 1.2.1.12) catalyzes the conversion of Glyceraldehyde-3-Phosphate (GAP) to 1, 3-Bisphosphate Glycerate (BPG) and plays a key role in glycolysis. The enzyme is involved in cellular processes such as apoptosis, membrane trafficking, iron metabolism and nuclear translocation. GAPDH (housekeeping gene) expression is stable and constitutive. Deregulation of GAPDH activity is associated with abnormal cell proliferation and carcinogenesis. Accurate quantitation of GAPDH activity is important for diagnosing diseases and studying normal cellular physiology. BioVision's GAPDH Activity Assay Kit provides a simple and sensitive method for monitoring GAPDH activity in various samples. In this assay, GAPDH catalyzes conversion of GAP into BPG and an intermediate, which reacts with a developer to form a colored product that absorbs maximally at 450 nm. Our high-throughput adaptable assay can detect GAPDH activity as low as 100 µU/mL in a variety of samples.



II. Application:

- Measurement of GAPDH activity in various tissues and cells
- Analysis of glycolysis and pentose phosphate pathways

III. Sample Type:

- Animal tissues: Liver, Heart etc.
- Cell culture: Adherent or suspension cells.

IV. Kit Contents:

Components	K680-100	Cap Code	Part Number
GAPDH Assay Buffer	25 ml	WM	K680-100-1
GAPDH Substrate	Lyophilized	Blue	K680-100-2
GAPDH Developer	Lyophilized	Red	K680-100-3
NADH Standard (500 nmol)	Lyophilized	Yellow	K680-100-4
GAPDH Positive Control	Lyophilized	Orange	K680-100-5

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA plate reader)

VI. Storage, Handling and Reagent Preparation:

Store kit at -20°C, protected from light. Read the entire protocol before performing the assay.

- **GAPDH Assay Buffer:** Warm to room temperature (RT) before use. Store at -20°C.
- **GAPDH Substrate:** Reconstitute with 220 µl of GAPDH Assay Buffer. Pipette up and down to dissolve completely. Keep on ice while in use. Aliquot and store at -20°C. Use within two months.
- **GAPDH Developer:** Reconstitute with 220 µl ddH_2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **NADH Standard:** Reconstitute with 400 µl ddH_2O to generate 1.25 mM (1.25 nmol/µl) NADH Standard solution. Keep on ice while in use. Aliquot and store at -20°C. Use within two months.
- **GAPDH Positive Control:** Reconstitute with 100 µl ddH_2O and mix thoroughly. Keep on ice while in use. Aliquot and store at -70°C. Use within two months.

VII. GAPDH Activity Assay Protocol:

1. **Sample Preparation:** For whole cells (1 x 10^6) or tissues (~10 mg), rapidly homogenize with 100 µl GAPDH Assay Buffer, and keep on ice for 10 min. Centrifuge at 10,000 x g, 4°C for 5 min. and collect the supernatant for the assay. Add 1-50 µl sample per well, adjust final volume to 50 µl with GAPDH Assay Buffer. Add 2-20 µl of GAPDH Positive Control into wells and adjust final volume to 50 µl with GAPDH Assay Buffer.
   Notes:
   a. For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve linear range.
   b. For samples having background, prepare parallel sample wells for sample background controls.
2. **NADH Standard Curve:** Add 0, 2, 4, 6, 8 and 10 µl of 1.25 mM NADH Standard into a series of wells in 96 well plate to generate 0, 2.5, 5.0, 7.5, 10 and 12.5 nmol/well of NADH Standard. Adjust volume to 50 µl/well with GAPDH Assay Buffer.
3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl reaction mix containing:

	Reaction Mix	Background Control Mix*
GAPDH Assay Buffer	46 µl	48 µl
GAPDH Developer	2 µl	2 µl
GAPDH Substrate	2 µl	----

Add 50 µl of the Reaction Mix to each well containing the Standards, Positive Control and test samples.

*For samples having high background, add 50 µl of Background Control Mix to each well and mix well.

4. **Measurement:** Measure the plate at 450 nm in kinetic mode for 10-60 min. at 37°C.

   Note: Incubation time depends on the GAPDH activity in the samples. We recommend measuring the OD in a kinetic mode and choosing two time points (T_1 & T_2) in the linear range to calculate the GAPDH activity of the samples. The NADH standard curve can be read in End point mode (i.e. at the end of sample incubation time).

5. **Calculation:** Subtract the 0 Standard OD value from all Standard readings. Plot the NADH Standard Curve. Subtract the background control OD value from all sample readings. Calculate the $\Delta OD = A_2 - A_1$ for the GAPDH activity of the test sample. Apply the ΔOD to the NADH standard curve to get B nmol of NADH generated by GAPDH activity during the reaction time ($\Delta T = T_2 - T_1$).

Sample GAPDH Activity = B/(ΔT X V) x D = nmol/min/µl = mU/µl = U/ml

Where: **B** is the NADH amount from standard curve (nmol)
ΔT is the reaction time (min.)
V is the sample volume added into the reaction well (µl)
D is dilution factor

GAPDH activity can also be expressed as U/mg of total protein in the sample.
Unit Definition: One unit of GAPDH is the amount of enzyme that will generate 1.0 µmol of NADH per min. at pH 7.2 at 37°C.

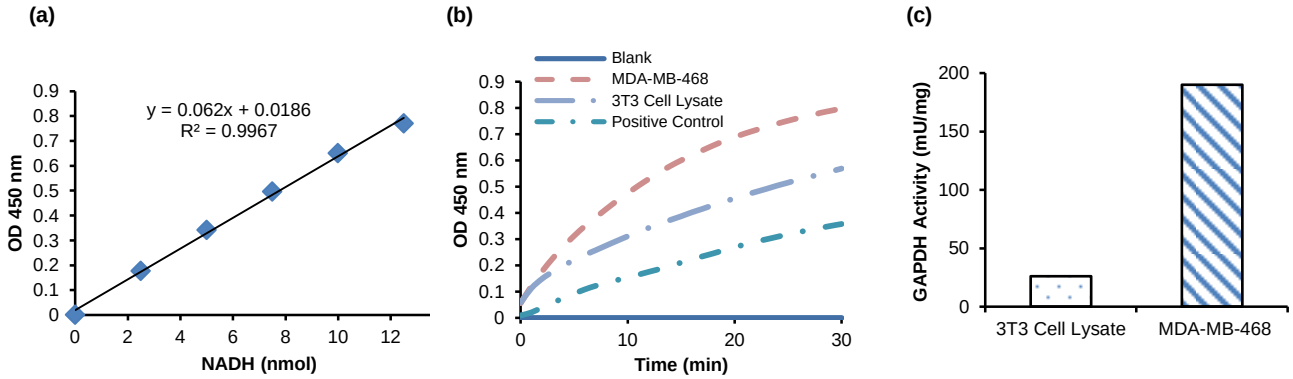


Figure 1: (a). NADH Standard Curve. (b). GAPDH activity in the Positive Control, 3T3 cells lysate and MDA-MB-468 cells lysate. (c). GAPDH specific activity calculated from 3T3 cell lysate (11.1 µg protein), and MDA-MB-468 cell lysate (2.76 µg protein). Assays were performed following kit protocol.

VIII. RELATED PRODUCTS:

Fumarate Colorimetric Assay Kit (K633)
Pyruvate Colorimetric /Fluorometric Assay Kit (K609)
Pyruvate Dehydrogenase Activity Assay Kit (K679)
Succinate (Succinic Acid) Colorimetric Assay Kit (K649)
Succinate Dehydrogenase Colorimetric Assay Kit (K660)
Alpha-Ketoglutarate Colorimetric Assay Kit (K677)
Malate Colorimetric Assay Kit (K637)
Malate Dehydrogenase Activity Assay Kit (K645)
Succinyl CoA Synthetase Assay kit (K597)
Isocitrate Colorimetric Assay Kit (K656)
Isocitrate Dehydrogenase Activity Assay Kit (K756)
Aconitase Activity Colorimetric Assay Kit (K716)

FOR RESEARCH USE ONLY! Not to be used on humans.